in between, with the upper helix capped by GAAA tetraloop. The NMR titration of magnesium ions with D5 indicates that it act as a potential metal binding platform. Here we are reporting the structure of a deletion mutant of D5 which is defective in catalysis of the substrate, but effective in binding to D123 domain.

**1297-Pos Board B207**
**The Multiple Substrate Recognition Properties of Ribonuclease P: Achieving Uniformity in Processing Kinetics Despite Variation in Substrate Structure**
**Lindsay E. Yandek**, Michael E. Harris.
Ribonuclease P is an essential enzyme that is responsible for catalyzing the maturation of the 5' end of transfer RNA. In Bacteria, the RNase P holoenzyme is composed of a RNA subunit and a protein subunit. The RNA subunit of RNase P contains the enzyme active site and has the ability to process substrates in the absence of the protein component *in vitro*, but works as a holoenzyme *in vivo*. While nucleotide recognition elements adjacent to the site of processing have been identified, different tRNA sequences vary considerably. Remarkably, rates of pre-tRNA processing are uniform despite this variation in substrate sequence and structure. The mechanistic basis for multiple substrate recognition by the holoenzyme is the focus of this study, with the ultimate goal being a better understanding of uniformity in ptRNA processing and discriminates between cognate and non-cognate RNAs. We are determining kinetic schemes for a number of pre-tRNAs using fluorescence assays and standard discontinuous assays. Multiple turnover kinetics have been obtained for ptRNA608$^{MET}$, a consensus pre-tRNA, and ptRNA605$^{f\text{-}MET}$ a non-consensus sequence. Both ptRNAs display a similar $V_{max}$, and Km values. Neither tRNA displayed any burst nor lag phases in pre-steady state kinetics implying that the rate limiting step for our processing model is catalysis. Our initial hypothesis is that uniformity in substrate kcat/Km values results from differential 5' leader sequence interactions with the protein that compensate for deviations from tRNA consensus recognition sequences. To obtain more insight into sequence and structure influence, we have set up a series of multiple turnover experiments with a consensus and non-consensus ptRNA competing against one another.

**1298-Pos Board B208**
**Measuring the Dimensions of a Compact Kinetic Intermediate in the Folding Pathway of the GlmS Ribozyme**
**Steve Meisburger**, Krista Brooks, Suzette Pabit, Li Li, Joshua Blose, Ken Hampel, Lois Pollack.
Using complementary time-resolved biochemical and x-ray probes of RNA structure in solution, we investigate the cation-induced folding of the glmS ribozyme, a metabolite-sensing RNA switch that regulates gene expression in bacteria. Hydroxyl radical footprinting experiments have shown a concerted folding transition within the first 10 seconds after adding magnesium. From small angle x-ray scattering (SAXS) experiments performed under similar conditions, we find that native tertiary contact formation is preceded by the collapse of the molecule to a relatively compact intermediate. The subsequent compaction observed by SAXS correlates temporally with changes in hydroxyl radical protection. We propose a structural model for the intermediate and possible implications for the role of secondary structure and electrostatics in the folding process of this ribozyme.

**1299-Pos Board B209**
**Folding Kinetics for the Conformational Switch Between Alternative RNA Structures**
**Song Cao**, Boris Furtig, Harald Schwalbe, Shi-Jie Chen.
The conformational switching between different conformational states is intrinsic to RNA catalytic and regulatory functions, which oftenly occurs on time-scales of several seconds. In combination with the recent real-time NMR experiments (Wenter et al. Angew. Chem. Int. Ed. (2005). 44, 2600; Wenter et al. ChemBioChem. (2006). 7, 417) for the transitions between bistable RNA conformations, we combine the master equation method with the kinetic cluster method to investigate the detailed kinetic mechanism and the factors that govern the folding kinetics. Based on the computational studies, we propose that heat capacity change upon RNA folding may be important for RNA folding kinetics. In addition, we find that noncanonical (tertiary) intraloop interactions in tetraloop hairpins are important to determine the folding kinetics. Furthermore, through theory-experiment comparisons, we find that the different rate models for the fundamental steps (i.e., formation/disruption of a base pair or stack) can cause contrasting results in the theoretical predictions.

**1300-Pos Board B210**
**Structure and Dynamics of "SWITCHING" RNAs**
**T. Kwaku Dayie**.
To date, most RNA structural information have been derived almost entirely from either X-ray crystallography or NMR spectroscopy. Even though X-ray crystallography has no size limit, there are concerns about potential crystal packing artifacts and a large number of RNAs remain refractory to crystallization. NMR is uniquely placed to tackle those classes of macromolecules that resist crystallization. To play an effective role, however, at least two inherent limitations of NMR that include signal overlap and rapid signal decay need to be circumvented. In the past, uniform isotopically enriched RNA samples have been used to determine the structures of small (<30 nucleotides) RNAs by NMR. However, as RNAs become larger and more interesting, the proton and carbon spectra of uniformly labeled RNA samples become a tangled web of overlapping peaks, severely limiting the usefulness of NMR. As an effective workaround, we have developed an array of new selective labeling schemes based on biomass nucleotide production using various bacterial strains, and we have designed new NMR experiments to exploit these labels. To test the hypothesis that some riboswitches employ conformational structural rearrangements to sequester the Shine-Dalgarno sequence in response to metabolite binding, we have initiated NMR studies of ligand-free riboswitches using our selective labels. We show that in the absence of ligands, there is a small population of G-C Watson-Crick base pairs, suggesting that the ligand-free riboswitch, though partially folded, samples some of the folded conformation in solution. Our methodology should be broadly applicable to other RNAs that require "switching" for their function.

**1301-Pos Board B211**
**Ligand Induced Conformational Changes of Riboswitches Probed by SAXS and NMR Spectroscopy**
**Bin Chen**, Xiaobing Zuo, Yunxing Wang, T. Kwaku Dayie.
Ribsowitches are a newly discovered large family of structured RNA elements that are typically located at 5' untranslated regions of messenger RNAs. They are composed of two domains: aptamer domain and expression platform. The aptamer domain functions as a sensor to recognize and bind specifically cellular metabolites while expression platform modulates gene expression in response to the conformational changes in the aptamer by either inhibiting transcription elongation or blocking translation. More than twenty riboswitch subfamilies have been identified so far in a variety of organisms ranging from bacteria, fungi to plants, indicating that riboswitch is a widespread genetic control element. Structural studies of ligand-bound riboswitches by X-ray crystallography and NMR spectroscopy provide insight into detailed RNA/ligand recognition and interactions. The structure, however, of ligand-free riboswitches remains poorly characterized. For better understanding the mechanism of riboswitches' functions and their transition from ligand-free and ligand-bound forms, it is critical to characterize the molecular details of the unliganded state. We have employed a variety of biochemical, biophysical, and computational techniques including SAXS and NMR spectroscopy to characterize the ligand-free and ligand-bound forms of riboswitches. Our data reveal that ligand binding of the RNAs causes significant conformational change and that only after ligand-binding does the formation of various secondary and tertiary structural elements occur.

**1302-Pos Board B212**
**Exploring Wild-Type and Mutant E. coli Strains for the Synthesis of Site-Specific Labels to Study RNA Structure and Dynamics by NMR**
**Jacob N. Sama**.
Ribonucleic acids are involved in many biological processes including catalysis, transfer and translation of genetic material, and regulation of gene expression. This unique ability to perform a variety of functions, traditionally associated with proteins is largely due to their capacity to adopt three-dimensional structural folds. Studying the 3D architecture of RNAs is critical for not only understanding the molecular basis of RNA function, but will eventually help with structure-assisted drug design, discovery and delivery. Heteronuclear NMR has become a powerful tool for studying the structure and dynamics of RNAs. To date, several RNAs have been well characterized by this method. However, overcrowding of chemical shifts and rapid signal loss in larger RNAs renders current NMR methods ineffectual. To study the structure and dynamics of larger RNAs, the use of site-selectively 13C-labeled nucleotides promises to be very helpful. To synthesize these labels, it was hypothesized that the metabolic pathways of various Escherichia coli wild type and mutant strains are capable of producing specifically labeled nucleotides necessary for making RNA. To test this hypothesis, we evaluated the growth of mutant strains K10-1516 (deficient in glucose-6-phosphate dehydrogenase of the pentose phosphate pathway) and DL323 (deficient in the Krebs